Discussion. Removal of the pineal gland from 9-week-old rats (Table I) resulted in an increase in pituitary MSH content which occurred within 3 days and appeared to persist at least 1 month. In 3-week-old rats a significant elevation of pituitary MSH levels was also observed in 2 out of 3 experiments 3 days after pinealectomy. However, this elevation disappeared within 1 week after removal of the pineal from these younger rats.

Melatonin has been shown to decrease pituitary MSH content(3). Conversely, MSH-release inhibiting factor (MIF) of hypothalamic origin increases levels of pituitary MSH(9,10). It might be reasonable to assume, therefore, that pinealectomy resulted in elevation of pituitary MSH content because of the absence of the stimulatory influence of melatonin or other pineal substances. Pinealectomy thus would be considered to have removed the mechanism which opposed the action of MIF.

The adjustment of pituitary levels of MSH toward control values may have involved, in addition to MIF, changes in circulating MSH(3) or a possible MSH-releasing factor (9,11). The feedback action of circulating MSH, however, may be mediated by MIF(3, 12). The results indicate that the compensatory adjustment following pinealectomy probably involved MIF and occurred more rapidly in the younger rats (Table II) than in the older ones (Table I).

Summary. Pinealectomy resulted in elevation of the MSH content of the pituitary glands of albino rats. This increased level of pituitary MSH persisted for several weeks in 9-week-old rats, but for only a few days in 3-week-old rats.

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Protection Against Lethality of Endotoxin with Isoproterenol: the Chick Embryo as an Experimental Model.* (31987)

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The 10-day chick embryo is very susceptible to endotoxin when administered by one of several routes. Protection against endotoxin is obtained with adrenocorticosteroids but not with heparin or phenoxybenzamine(1,2). Experimental and clinical evidence indicates that Isoproterenol is effective in endotoxin shock(3-7). In the present studies the chick embryo was used as a model to quantitate the lethality of intravenously administered endotoxin and protection of Isoproterenol.

Methods. White leghorn fertile eggs (Ghostley Pearl, Anoka, Minn.) received prior to incubation were used throughout. Ten-day eggs were candled, a large vein

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TABLE I. Lethal Effect of Endotoxin in the 10-Day Chick Embryo.

Endotoxin, μg	Alive	Dead
.1	1	19
.05	35	147
.01	41	5

selected, and the shell marked to indicate direction of blood flow. A triangular shaped portion (7 × 7 × 7 mm) of the shell with the apex pointing in the opposite direction of venous flow was cut with a dental drill and removed. A drop of sterile mineral oil was placed on the exposed membrane permitting good visualization of the vessel when transilluminated. A total of 0.1 ml of solution was injected intravenously. Saline controls were used in all experiments. Eggs showing immediate evidence of traumatic bleeding were discarded. The eggs were placed in a humidified incubator set at 39.5°C for 18-24 hours. Viability was determined by candling and all questionable and non-viable embryos were examined grossly. Only those embryos with clear evidence of hemorrhage were recorded in the final results. Embryos appearing pale with associated evidence of extra fetal bleeding were excluded.

Materials. Endotoxin: A single lot of *Escherichia coli* endotoxin prepared in our laboratory as previously described(8) was used in all experiments. The same lot of injectable Isoproterenol was used.† Each ml of Isoproterenol contained 0.2 mg of isoproterenol hydrochloride, 7 mg of sodium chloride, 0.12 mg lactic acid, 1.8 mg sodium lactate, sufficient additional lactic acid or sodium lactate to establish a pH 4.5 to 5.0 range, and not greater than 1 mg sodium bisulfite as a preservative. The effect of these constituents was not determined independently. The syringes were monojet 501-T tuberculin, sterile, non-toxic, non-pyrogenic, fitted with 27 gauge needle.‡ The egg incubator was humidified and temperature controlled.§

Results. The mortality rates with varying amounts of endotoxin indicated that 0.05 μg of endotoxin was most appropriate for study purposes since only 35 out of 182 embryos survived (Table I). Embryos tolerated Isoproterenol well, 27 of 28 embryos surviving 2.0 μg and 21 of 23 surviving 20.0 μg.

A series of 8 different experiments involving 415 embryos demonstrated the protective action of various doses of injectable Isoproterenol against 0.05 μg of endotoxin when both materials were simultaneously administered intravenously (Table II). Lethality from endotoxin alone varied from 56 to 91%. Remarkable protection against endotoxin was obtained with as little as 0.1 μg of Isoproterenol. Amounts of 0.1 μg of the drug were as effective as 10 μg. The consistency of protection with 0.1 μg Isoproterenol against 0.05 μg endotoxin is demonstrated in experiments 4, 5, 6 and 7 where the p values as determined by the x^2 test of independence were <.002, .001, <.001 and .001, respectively. These are highly significant. Protection was not obtained with 0.01 μg of Isoproterenol where the p value of .269 is not significant.

Discussion. The pathogenesis of endotoxin shock in experimental animals and in human patients remains unclear. While the chick embryo does provide an effective and

TABLE II. Protection with Varying Doses of Isoproterenol Against Endotoxin in the Chick Embryo.

Exp	Endotoxin, μg	Isoproterenol	Alive	Dead	p value*
1	.05		4	41	<.001
	.05	+ 10 μg	40	8	
2	.05		5	24	<.001
	.05	+ 5 "	25	2	
3	.05		7	15	<.001
	.05	+ 1 "	23	1	
4	.05		10	15	<.002
	.05	+ .1 "	21	4	
5	.05		3	18	.001
	.05	+ .1 "	12	6	
6	.05		2	16	<.001
	.05	+ .1 "	15	4	
7	.05		2	18	.001
	.05	+ .1 "	12	8	
8	.05		12	15	.269
	.05	+ .01 "	8	19	

* p value determined by x^2 as a test of independence.

† Winthrop Laboratories, New York.

‡ Roehr Products Co., Norfolk, Neb.

§ Montgomery Ward Model #052Z-3665B.

relatively inexpensive living model for screening potential therapeutic agents, meager information is available on the nature of endotoxin shock in the embryo. The susceptibility to endotoxin has been ascribed to the release of catecholamines from the adrenal medulla (1). Associated with this is the undeveloped function of the adrenal cortex so that a deficiency of endogenous glucocorticoid and susceptibility to endotoxin can be surmounted by administering the hormone, or one of its analogues. Cationic cyclic polypeptide antibiotics such as Polymyxin B sulfate, Colistin sulfate and tyrocidine hydrochloride have been reported to protect embryos against lethal doses of endotoxin(9). Finkelstein(2) pointed out that endotoxin causes hypoglycemia and death in the embryo, which can also be accomplished by injecting 4 μg of insulin. It had been postulated that circulatory stasis caused by "sludging" of blood led to the hypoglycemia but heparin did not alter the outcome, which has been confirmed in our laboratory. Other agents reported not to effect endotoxin lethality are cholesterol, deoxycorticosterone, l-dehydrocortisone, heparin and phenoxybenzamine. Although phenoxybenzamine does not protect against endotoxin in the embryo, this adrenergic blocking agent does protect against a lethal dose of epinephrine or norepinephrine(2).

The protection provided by Isoproterenol in the embryo centers attention on the role of adrenergic substances in endotoxin activity. Isoproterenol is a β-adrenergic stimulator having both inotropic and chronotropic actions, induces vasodilation and hyperglycemia, and affects other autonomic end organs(10). Although embryos tolerate large doses of Isoproterenol without apparent ill effect the mechanism of action in the chick embryo against endotoxin remains unknown.

Summary. The susceptibility of the chick embryo to endotoxin is confirmed. Embryos tolerate up to 20 μg of Isoproterenol when injected intravenously. Isoproterenol in amounts of only 0.1 μg significantly protect embryos against 0.05 μg of endotoxin, which is greater than an LD_{50} dose. The mechanism of the protective action of Isoproterenol is not known.

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Increased Resistance to Endotoxin in Germ-Free Guinea Pigs.* (31988)

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Reactivity to bacterial endotoxins can be modified not only by experimentally acquired infection with various heterogeneous microorganisms(1) but also by the type of bacteria present in the gastrointestinal tract. Schaedler and Dubos(2) found that Rocke-

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